399

SOME EFFECTS OF DDT ON THE GUPPY AND THE BROWN TROUT

SPECIAL SCIENTIFIC REPORT–FISHERIES No. 399

UNITED STATES DEPARTMENT OF THE INTERIOR
FISH AND WILDLIFE SERVICE

EXPLANATORY NOTE

The series embodies results of investigations, usually of restricted scope, intended to aid or direct management or utilization practices and as guides for administrative or legislative action. It is issued in limited quantities for official use of Federal, State or cooperating agencies and in processed form for economy and to avoid delay in publication.

UNITED STATES DEPARTMENT OF THE INTERIOR, STEWART L. UDALL, SECRETARY
Fish and Wildlife Service, Clarence F. Pautzke, Commissioner
Bureau of Sport Fisheries and Wildlife, Daniel H. Janzen, Director

SOME EFFECTS OF DDT ON THE GUPPY AND THE BROWN TROUT

By

Susan Frances King

United States Fish and Wildlife Service
Special Scientific Report--Fisheries No. 399

Washington, D. C.
March 1962

CONTENTS

Editor's Note: This paper was prepared under the direction of Assistant Professor Kathryn M. Eschenberg, Mount Holyoke College and was accepted by the Faculty in partial fulfillment of the requirements for the degree of Bachelor of Arts with Honor, 1961. Despite certain limitations, described below, the paper (somewhat condensed from the original) has been accepted for publication because (1) it is an unusual and excellent study by an undergraduate, and (2) so little information is available on the subject treated. Biometricians may criticize the paper from the standpoint of small numbers and need for replication. One reviewer says: "One of the most interesting observations related to the apparent increase in resistance following exposure to effectively sublethal levels. Part of the increase, but likely only part, was due to the previous selective killing of weaker individuals. Good experimental procedure would require that this test be repeated, with parallel series of previously unexposed fish....There is need for a replicated study of numbers of young born to guppies held in DDT, especially because the numbers are so small." It is the Editor's hope that the limitations of the paper may stimulate further work in this important area.

SOME EFFECTS OF DDT ON THE GUPPY AND THE BROWN TROUT

By

Susan Frances King[1/]

Since the discovery of DDT's insecticidal properties in 1942, pesticide production has mushroomed to over two hundred basic types prepared in more than six thousand formulations. These pesticides have been used extensively in the control of plant and animal pests and disease carriers. The chemical agents used to control insects are, however, deleterious to higher forms of life as well. As a result, precautionary measures of pesticide application have evolved along with the development of the pesticides. Nonetheless, after application of the chemicals reports of destruction of fish and wildlife are frequently received by the conservation offices.

DDT production constitutes at least 31 percent of total pesticide production (Annon., 1960). Its uses at present outnumber the uses of the other chlorinated hydrocarbons and it has proven most valuable as a means of controlling forest pests. DDT was one of the first insecticides to be studied by conservationists in relation to the effects on fish and wildlife. Evidence of the toxicity of DDT, especially to fishes, determined by field studies and laboratory bioassays, has accumulated in the literature. It has been ranked fifth in toxicity to fish in a series of nine of the most commonly used chlorinated hydrocarbons (Henderson et al., 1959a). Endrin, toxaphene, dieldrin, and aldrin outrank it in toxicity and heptachlor, chlordane, methoxychlor, and lindane are somewhat less toxic. Much of the early data toxicity, especially that concerning fish, is incomplete and of little practical application, since vital information such as environmental conditions is often lacking (Cottam et al., 1946; Nelson et al.,1947; Adams et al.,1949; Ginsberg et al.,1954). More extensive studies of effects of insecticides in the field may be found in the works of Surber (1951), Ingram and Tarzwell (1954), Kerswill and Elson (1955), Shepard (1956, et al., 1959), Leffler (1958), Tarzwell (1958), and Keenleyside (1959).

Some of the first laboratory bioassays on fish were conducted by Surber (1947), Linduska and Surber (1948), and Lawrence (1950). Surber and Lawrence found a vast difference in toxicity levels of insecticides in the laboratory and in the field. The toxicity of DDT to bluegills in the laboratory was .14 ppm (parts per million) and .04 ppm in the field. Even the tolerance level median (TL/m) reported in the literature by different workers for the same species of fish is found to vary considerably. Surber designated .14 ppm and Henderson et al. (1959a) .021 ppm DDT as toxic concentrations to bluegills. Henderson has conducted brief bioassays following standardized procedure on fatheads, bluegills, goldfish, and guppies with ten different chlorinated hydrocarbons, including DDT in hard and soft water and in various formulations. Each species varied considerably in sensitivity to each compound. The TL/m in ppm DDT in acetone solution for 96 hours for guppies was .043; for fathead minnows, .032; goldfish, .027; and bluegill, .016. Tarzwell and Henderson (1957) have studied effects of dieldrin, a chlorinated hydrocarbon similar to DDT in chemical structure and toxicity effects, on fatheads, small bluegill, and green sunfish.

Until recently, little consideration was given to the more extensive effects which DDT might have on growth and reproduction of large organisms. Allison (Fishery Research Biologist stationed at the National Fish Hatchery, Jackson, Wyoming) is beginning long term exposure of cutthroat trout to DDT in bath form and in the diet, to study growth and reproduction. In addition, studies to understand effects of size, sex, physical conditions, dosage rate, water chemistry, and environment on toxicity are in progress at the Denver, Colorado, Fish-Pesticide Research Laboratory (DeWitt et al., 1960). Studies of effects

1/ Present address: Duke University, Durham, N.C.

of repeated sublethal doses of pesticides on survival, growth, and reproductive potential, through at least one reproductive cycle and with the use of histology and hematology as tools are also in process there (vonLimbach, 1960[2/]). Some studies of inhibitory effects of DDT on reproduction of quail, pheasants, and dogs (DeWitt, 1955, 1956; Rudd et al., 1956a, b; and Kitselman, 1953, respectively) have already been reported, and a possibility of similar effects on fish and wildlife seems unquestionable.

A standardized procedure and means of interpreting data were found to be essential for comparing results of different workers, and, to avoid the discrepancies in toxicity values reported by the first investigators in this field. The first effort to standardize bioassay methods was made by Hart et al. (1945). This procedure, simplified by Doudoroff et al. (1951, in affiliation with the toxicity subcommittee of the Federation of Sewage and Industrial Wastes Association), is followed by most industries which test the toxicity of their wastes on fish (U. S. Public Health Service, 1956, 1957). The method is simple and yields reliable, reproducible results.

The bioassay procedure involves placing the fish in serial dilutions of a toxicant and recording the percent survival in each concentration at specified times. The dilutions are generally in a logarithmic series for ease in plotting results. To express the effects of a substance on fish, a value termed the tolerance level median (TL/m) has been used by various workers (State Water Pollution Control Board, 1952; Tarzwell, 1957a, b). This value is the concentration of the substance under investigation in which 50 percent of the test animals are able to survive for a specified period of time under the conditions of the experiment.

Studies to determine the methods of action of DDT and other insecticides on fish and wildlife have also involved both biochemical and histological investigations. The chlorinated hydrocarbons such as DDT are readily concentrated in fish, especially in the fatty tissues (Garner, 1957; Cope, 1959), but seem to be of little harm to the fish in low concentrations. No complete survey of histopathological conditions occurring in fish exposed to chlorinated hydrocarbons was found in the literature, though fairly extensive work has been done by Baxter (1959) on lambs exposed to aldrin, by Kitselman (1953) on dogs exposed to aldrin and dieldrin, and by Bell (1961) on goats exposed to DDD. The external signs of stress in animals exposed to some of the chlorinated hydrocarbons, and histopathological conditions involving particularly the degeneration of the zona fasiculata of the adrenal gland and severe liver and intestinal disturbances have lead various workers to suggest a direct inhibitory effect of the insecticides on adrenal tissue (Nelson et al., 1949; Gowdey et al., 1955; and Bell, 1961). Modification of the normal enzyme system and metabolic pathways by insecticides is being investigated at present by Hosein (reported by Weiss, 1960).

The present study was undertaken to determine the effects of chronic exposure of DDT on fish, using the guppy (Lebistes reticulatus) as the basic laboratory test animal and correlating results with those obtained using the local brown trout fry (Salmo trutta).

Henderson et al., (1957) state, "While they (guppies) are not of economical or recreational importance in receiving waters and biological assay results may not be applied to other fish directly, guppies are among the most desirable form from the standpoint of maintenance in the laboratory and uniformity of available stock. If provision is made for comparison with important local species under similar conditions, the guppy may be considered a .desirable test fish for routine biological assay work."

Guppies (Lebistes reticulatus) belong to the Poeciliidae, a family of live bearers. They

2/ vonLimbach, B. 1960 Communication of August 2, 1960, to Chief, Fish-Pesticide Research Laboratory

are moderately resistant to polluted waters (Hart et al., 1945), and are adaptable to laboratory conditions of temperature, food, and handling. Their small size and availability are also important factors. They thrive under crowded conditions, are inexpensive, and can tolerate temperatures from 65° to 100° F. (U. S. Fish and WildlifeService, 1960). For the experiments under consideration, the small size, and rapid growth and reproductive rates were primary factors in the choice of the guppy as a test fish. They have also been used by other workers in bioassay studies (Warren et al., 1958; Henderson et al., 1959a, b).

In order to determine sublethal dosages of DDT, 14 day bioassays were conducted under standard conditions using trout and guppy fry and adult guppies. Once the sublethal dosage for the adult guppy was determined, a series of sublethal dilutions of DDT was set up to observe the guppies over a more extended period of exposure to DDT. Less extensive studies were conducted to observe the possible effects of the DDT on growth and survival of guppies born in the toxicant and the effects of gradually increased concentrations on sensitivity or resistance to the DDT. The brain, liver, kidney, and intestine of the trout fry and adult guppies were prepared for histological examination in order to determine the histopathological conditions resulting from the presence of DDT in the organism.

MATERIALS AND METHODS

The bioassay procedures in this study were conducted in accordance with the standardized methods as far as they were applicable. A stock solution of 250 milligrams technical DDT[3/] in 25 cc acetone solvent (Fisher certified reagent) was used for all the tests (recommended by Cope et al., 1947).

The test water for bioassays is of particular importance. It must be ideal for the fish in the absence of the toxicant under investigation. The water used in all the bioassays came from a deep well near the Connecticut River by South Hadley, Massachusetts. Use of this water source avoided the fluctuation of water characterlstics such as pH, alkalinity, and hardness which occurs in city water(Farris, 1950). Well water such as this which lacks high mineral content is considered satisfactory for guppies (U. S. Fish and Wildlife Service, 1960). The pH of the water was 8.4. A pH of 7 is ideal for trout, while a range of 6.7 - 8.6 is recommended for guppies (Turner, 1937).

In order that gases in the water might come to equilibrium with gases in the atmosphere, the water was allowed to stand in open glass vessels for a minimum of 7 days before a test was begun. To offset any possible decrease in oxygen level which might result from the addition of acetone (Henderson, et al., 1959a) air was vigorously pumped through the water for at least one hour before the tests. Carbon dioxide level was not measured since this gas remains at a sufficiently low level if the water contains sufficient oxygen.

Bioassays with the guppy

In the experiments involving adult guppies, light, temperature, food, water, oxygen, and sex ratio were as advocated by Gordon (1955; also Silliman, 1948; U. S. Fish and Wildlife Service, 1960; Breder et al., 1932; Gibson et al., 1955). Fifteen watt candelabra light bulbs were suspended 7-1/2" above the water in the aquaria and turned on approximately 13 hours a day. Temperature, checked at least twice daily, averaged between 23° and 25° C., ideal for guppies, with variations for short periods from 21° to 27° C. Though temperature is usually kept within a variation of ± 1° C in controlled experiments, a constant water bath was not available and the variation of ± 3° C was well within the temperature range in which guppies thrive (72° - 82° F). Food consisted of dried commercial aquarium food daily with white worms, Enchytraeus, twice a week. A varied

3/ Technical DDT, or $C_{14}H_9Cl_5$, is a white powder, soluble in most organic solvents, but not in water. It is 76 percent p, p'isomer, 25 percent o, p'isomer. The DDT used in the experiments came from a commercial supplier, the Diamond Alkali Company.

diet is considered desirable for best growth and breeding. The fish were fed all they could eat within a five minute period in the mornings, and excess food was removed to prevent clouding of the water. The fish were acclimatized to laboratory conditions for a minimum of 7 days before toxicity tests. No guppies were taken from tanks where losses were occurring.

To facilitate exchange of gases such as loss of carbon dioxide and uptake of oxygen at the surface, an electric air pump operated constantly, allowing air to enter each tank by means of glass tubing. The bubbling, though sufficient to keep the water slowly circulating, was limited to less than eight bubbles a minute in order to prevent spraying of water and consequent loss of DDT onto the glass covers and sides of the tanks. The allotted volume of water per fish was 700 cc/guppy. To prevent competition for oxygen, Turner (1937), Farris (1950), and Silliman and Gutsell(1958) recommended about 9 adult guppies per 6 liters or 660 cc/fish. The frequent removal of wastes was imperative as the presence of wastes in the water would increase the carbon dioxide level and chlorinated hydrocarbons are likely to adsorb to organic material. Therefore, two liters of water were siphoned from each bioassay test aquarium and filtered weekly.

One to 14-day TL/m determinations for adult guppies: -- To determine what concentrations of DDT to use in the two week test series, a wide range of concentrations -- 1.0, .56, .18, and .10 ppm (milligrams per liter) DDT were tested for a 24-hour period by placing two fish in 1400 cc of each concentration in wide mouth jars of 3-1/2 liter capacity. As one of the two survived in the .10 ppm for 24 hours and none survived the other concentrations, .10 ppm was the highest concentration tested in the 2-week tests.

Ten fish has been considered an adequate number for bioassays by Hart et al., (1945) and 10 guppies, 6 female and 4 male, were used. Duplicate samples of 5 fish were placed into 3-1/2 liter duplicate samples of DDT solutions, in museum jars 5-3/4" high and 8-1/2" in diameter. Concentrations of .10, .056, .032, .018, .010, .0056, and .0032 ppm of DDT had been added from the stock DDT solution within 30 minutes prior to the test. Records of percent survival were kept and dead fish removed as soon as possible. To determine the TL/m for one, 4, 7, and 14 days the recommended method of straight line graphical interpretation was used, plotting the percent survival of fish against the logarithmic concentration (fig. 1, form recommended by Doudoroff et al., 1951). As Hart noted, there are generally not enough points for a sigmoid or s-shaped curve and the straight line graphic interpolation is sufficient for practical purposes. The median is not influenced by extreme variance and 100 percent mortality criteria would not be a measure of tolerance.

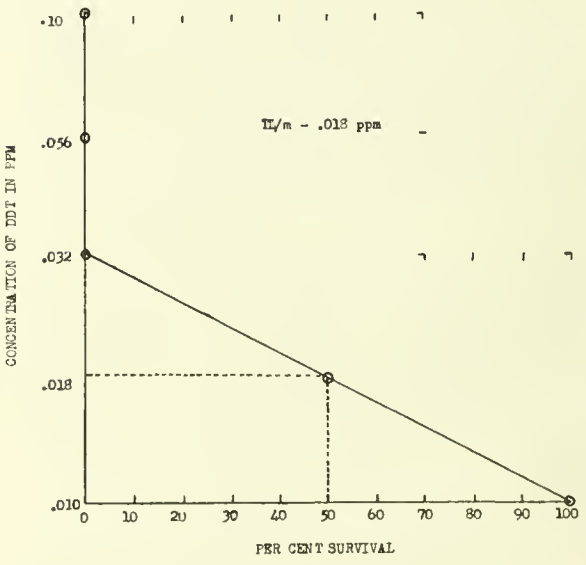

Figure 1:--Fourteen day TL/m determination for adult guppies exposed to DDT (data from table 1).

Resistance to .032 ppm DDT of two different strains: -- Even though the guppies were acclimatized for at least 7 days before being tested in DDT, it seemed possible that strains from varied backgrounds might differ in tolerance to the toxicant and a duplicate set of 5 fish from a different strain was exposed to .032 ppm DDT under constant conditions to compare results with the 14-day bioassay.

Resistance to DDT of fish exposed to sublethal dosages: -- To determine if a primary

exposure to a sublethal concentration of the insecticide increased or reduced the resistance of the fish to DDT, those adult guppies surviving the 2-week test series were transferred immediately after 14 days into tanks of freshly prepared concentrations of .032 ppm under constant conditions. Records on survival were kept for 30 days.

Stability of the stock solution: -- To ascertain if the toxicity of the stock solution remained constant, the stock which had been standing for two months was compared with a freshly prepared stock solution. Identical test solutions of .032 ppm in 3-1/2 liters of water were made from each stock to which duplicate sets of five normal fish from the same strain were added and observed for 14 days.

Thirty-day exposures to sublethal dosages of DDT with some observations on growth and reporduction: -- Conditions for extended tests varied from the 14-day TL/m determinations only by the use of two gallon "squash" aquaria which held 7 liters of test solution and 10 fish per tank. No duplicates were conducted. Concentrations of .0185, found previously to be the concentration in which at least 50 percent of the guppies could survive in a 2-week period, .010, .0056, .0018, and .001 ppm were tested and a control was set up, this time adding the same concentration of acetone to the control as used in the .0185 ppm tank (.013 cc acetone).

It was hoped that effect of DDT on weight could be determined and the fish from each tank were weighed, males and females separately, at the beginning of the experiment, and at two intervals of two weeks. The weight was determined by weighing the fish to the nearest 1/100 of a gram in 150 ml glass beakers with a minimum of water. To avoid excessive handling of the fish, measurements of length were not taken.

As a result of some disease or of an increasingly high level of dissolved wastes in the water, the fish started to die rapidly in each of the tanks including the control after 30 days, and the experiment was discontinued. A repeat of the test using sterilized glassware and filtering the water every 5 instead of 7 days ended also because of the unexplained deaths of the fish after 30 days.

An apparatus was designed to trap and separate young born in DDT from the adult guppies and consisted of a glass beaker 4" high in which was suspended a glass funnel covered with nylonized cloth. The beaker was set inside the larger aquarium under the usual experimental conditions. Gravid guppies were placed in the covered funnel, and the newly born fish settled through the stem of the funnel into the beaker below. Plastic traps were not used as the plastic reacts with and fouls the DDT test solutions, indicating possible chemical changes affecting the toxicity of the solutions.

Young guppies, born within a 2-day period 23 and 24 days after the experiment was begun, were placed separately in 3-1/2 liter widemouthed jars with 340 cc untreated well water per fish and kept under constant conditions of light, temperature, and food (dried aquarium food only) for 40 days. Measurements of length were taken at the end of this period.

One to 14-day TL/m determinations for 14-21 day guppy fry: -- Five 2- to 3-week-old guppy fry were placed in 1700 cc of water (340 cc/fish) in each of 9 widemouthed jars. DDT was added in concentrations of .056, .032, .018, .010, .0056, .0032, .0018, and .0010 ppm. Guppies of the same age in untreated well water served as controls. Light, temperature, and feeding conditions were kept constant. Only dried aquarium food was used. Daily records for survival rates were kept and any dead fish removed immediately.

Bioassays with young brown trout

In order to have conditions similar to those used on the adult guppies, 700 cc well water/fish were allotted in the trout assays. No mechanical aeration was used during the tests though the solution was aerated prior to the addition of DDT. The trout fry used in the bioassays and histological studies came from a local Massachusetts State Fish Hatchery and had been raised in running water of approximately 7° C. and a pH of 7. Before the tests, the fry were kept in tanks in non-circulating well water (pH 8.4) at approximately 8° C. Extended acclimatization to laboratory conditions was avoided before most of the following experiments

as it was feared that the trout might become less resistant in the still water.

One to 14-day TL/m determinations for 14-day trout fry with yolk sacs: -- Fourteen-day-old trout fry obtained from the hatchery two days prior to the experiment were exposed to serial dilutions of DDT in the following concentrations: 10, 3.2, 1.0, .56, .32, and .18 ppm DDT. Five fry were used for each concentration and were placed in 3-1/2 liters of the DDT treated water. Museum jars served as containers. The control consisted of five fish in untreated well water. Observations on percent survival and general physical reactions were recorded and dead fish removed daily. At the end of two weeks, the surviving trout fry were fixed in Bouin's fixative and prepared for histological examination.

One to 14-day TL/m determinations for 10-week trout fry: -- Ten-week-old trout fry, pigmented and without yolk sacs, were tested under conditions identical to those applied to the 2-week fry. The concentrations tested were .0056, .0032, .0018, .0010, .00056 ppm with a control of untreated well water. The trout had been kept in aquaria in the laboratory for 7 weeks before the experiment, had been fed medium sized tropical fish food since the fourth week, and were fed daily during the bioassay.

One to 14-day TL/m determinations for number one fingerlings: -- A bioassay on 11-week-old fish, averaging approximately 3.3 cm in length, was conducted two days after the fish arrived from the local hatchery. Conditions were similar to those in the previous trout assays except that 10 fish were used for each concentration instead of five and were kept in 2-gallon "squash" type aquaria. The fingerlings were regularly fed food pellets obtained from the hatchery. The concentrations tested were .032, .010, .0032, .0010, and .00032 ppm.

Histological study of trout fry and adult guppy tissues following exposure to DDT

Hematoxylin-eosin stained paraffin sections of Bouin's-fixed tissue cut at 8u were made of the kidney, liver, intestine, and brain of four male guppies, two of which were sacrificed after 24 hours and two after 48 hours of exposure to .032 ppm DDT. This concentration is toxic to adult guppies within 96 hours. Similar preparations were made from two normal guppies for controls. The fish were fed during the exposure to DDT. Cross sections of trout fry surviving a 14-day bioassay in 10, 3.2, and .56 ppm DDT, begun when the fry were two weeks old, were also prepared for histological study. Three normal trout of the same age served as controls.

RESULTS

Bioassays with the guppy

One to 14-day TL/m determinations for adult guppies: -- The TL/m values for adult guppies over a 2-week period were determined by graphical analysis to be .027, .0195, .0195, and .018 ppm for 2, 4, 7, and 14 days, respectively (table 1; refer to fig. 1 for the 14 day determination). As .018 was the TL/m for 14-day exposure and extrapolation of a time concentration curve of tolerance (fig. 2, form recommended by Hart et al., 1945) indicated that concentrations below this amount might be tolerated for an indefinite time, .0185 was the highest concentration used in the long range chronic exposure test. Symptoms of stress in response to DDT were hyperactivity, muscular convulsions, slight paling of pigmentation, followed by loss of balance and death. The time of onset of the symptoms varied with the concentration.

Table 1:--Survival of adult guppies in DDT solutions and TL/m determinations over a 2-weak period.

Concentration ppm DDT	Percent surviving after 2 days	4 days	7 days	14 days
.10	20	0	0	0
.056	30	20	10	0
.032	30	0	0	0
.018	100	60	60	50
.010	100	100	100	100
.0056	100	100	100	100
.0032	100	100	100	90
TL/m in ppm	.027	.0195	.0195	.018

Tolerance of .032 ppm DDT by two different strains: -- Placing a second strain of fish in .032 ppm gave results that indicated that strains from varying backgrounds differ

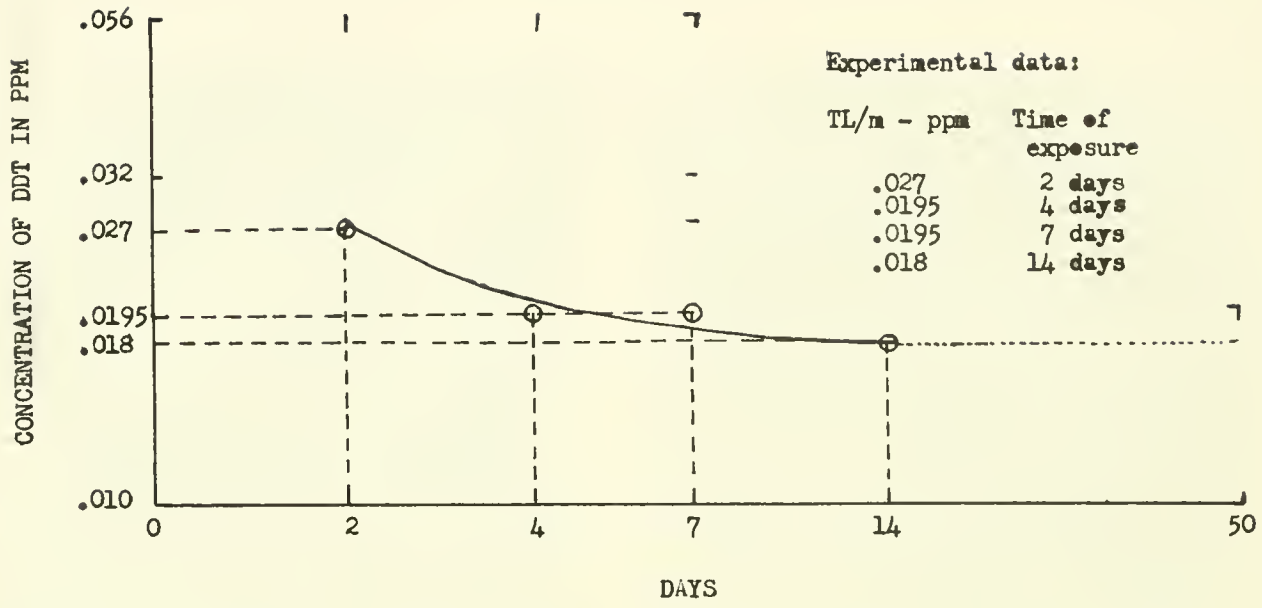

Figure 2: -- Time concentration curve of tolerance to predict a TL/m value of DDT sublethal to adult guppies under extended exposure based on two, 4, 7, and 14 day TL/m values.

in resistance to DDT. The first strain had been shipped from Florida, while the second strain came from a local source. The 14-day TL/m values for the two strains were .018 and slightly more than .032 ppm, respectively.

Resistance to DDT of fish exposed to sublethal dosages: -- The fish acquired increased resistance to DDT upon exposure to sublethal concentrations. On the basis of the limited number of fish tested (table 2), the fish in concentrations ranging from .0032 to .018 ppm for two weeks were more resistant to .032 ppm than unexposed fish. This concentration had proven lethal to normal fish of the same strain within three days, but appeared to have little effect on the DDT-exposed fish by this time, as some fish from each original concentration were still alive after three days in .032 ppm. Fish from .010 ppm and .0056 ppm were most resistant to the stronger dose of DDT since 80 percent and 60 percent, respectively, were still alive after 30 days. Twenty-two percent of those originally in .0032 ppm were alive after 30 days, while none from .018 ppm survived this long.

Table 2:--Survival of guppies transferred from sublethal concentrations to .032 ppm DDT.

Previous concentration in ppm	Number of fish	Percent survival in .032 ppm			
		3 days	7 days	15 days	30 days
.018	5	60	40	20	0
.010	10	100	100	90	80
.0056	10	80	80	70	60
.0032	9	88.9	44.4	33.3	22.2

Thirty-day exposures to sublethal dosages of DDT with some observations on growth and reproduction: -- As predicted by extrapolation of a time concentration curve of the 14-day TL/m values, two test series indicated that concentrations below .018 (.010, .0056, .0018, and .0010 ppm) were sublethal to 50 percent of the guppies, even over a 30-day period of exposure. Changes in weight were irregular and probably insignificant in the two tests. There was no marked loss or gain of average weight of fish in any of the tanks. In the first 30-day test, the total number of deaths in all the tanks was 6 out of 60 or 10 percent and occurred in 4 of the 6 tanks including the control. The death of these fish was attributed to the change of environment at the beginning of the experiment rather than from DDT poisoning.

Within 30 days after the beginning of the second extended test series, a total of 8 broods was born in the experimental tanks and the control (table 3). Out of 44 born in the DDT, 21 were born dead or died within several hours. Those born in .0185 and .010 ppm DDT (a total of 14) had abnormally large yolk sacs which were resorbed within several days if the fish survived. Six guppies of a brood born dead in .001 ppm DDT also had slightly distended yolk sacs. The twenty born later in this concentration were apparently normal.

Table 3:--Number and condition of guppies born in sublethal dosages of DDT.

Days since the beginning of the experiment	Concentrations of DDT in ppm											
	.0185		.010		.0056		.0018		.0010		control	
	A	D	A	D	A	D	A	D	A	D	A	D
21									6*			
22			4*	1*	3*							
23				6*				4		9		
24											5	
27										3	8	

* Distended yolk sac present; A=alive; D=dead

Preliminary observations were made on relative survival and growth rates of the DDT-exposed and control newborn. By placing the young guppies born in .010 and .001 ppm and in the control on days 23 and 24 of the above experiment into untreated well water, it was possible to obtain a tentative idea of the comparative survival and growth rates. The survival rate of the fish born in DDT was lower than that of those from the control. Of the 8 fish removed from .0010 ppm, the 6 from .010, and the 5 from the control, there were 4 survivors from each at the end of 40 days. The average total length of the guppies from the DDT test solutions was 10 mm while that of the guppies born in untreated water was 11 mm. The observations were made on limited numbers of animals however, and definite conclusions are unwarranted.

One to 14-day TL/m determinations for 14-21 day guppy fry: -- The sensitivity of 2- to 3-week guppies was considerably greater than that of the adults. While the 14-day TL/m for the adults was .018 ppm, it was as low as .0024 ppm for the young, almost a ten-fold difference (table 4). The TL/m values for the shorter periods of time were also consistently lower for the young than those for the adults. Stress symptoms were similar to those of adult guppies.

Table 4:--Summary of TL/m values for the young of the brown trout and young and adult guppies in 14-day bioassays

Kind of fish and age	TL/m values for DDT in ppm			
	1 day	4 days	7 days	14 days
Guppies				
2-3 weeks	.0158	.0075	.0029	.0024
adult	>.10 but <.56	.0195	.0195	.018
Trout				
2 weeks	>3.2*	>3.2*	2.4	<.18
10 weeks	.0057		.00195	.00056
no. one fingerlings (11 weeks)	.018	.0175	.014	.014

* Concentrations above 3.2 precipitated out of solution so values above 3.2 ppm could not be determined under present condtions of exposure.

Bioassays with young brown trout

One to 14-day TL/m determinations for 14-day trout fry with yolk sacs: -- As the TL/m values in table 4 for 14-day trout fry suggest, sensitivity to DDT increased markedly during the second week of exposure. From the seventh to fourteenth day, the fry became increasingly sensitive to the DDT and the survival dropped from 80 percent in the concentrations of 3.2 and .32 ppm and from 100 percent in .18 ppm on the seventh day to 0 percent in all three tanks by the fourteenth day. It was not possible to determine the precise TL/m value for the 96 hour period as the DDT precipitated out of solution in the concentration tested above 3.2 ppm (10 ppm).

The typical signs of stress in the DDT solutions were first a loss of balance, evidenced by the fish remaining on their sides rather than upright, and then almost complete loss of muscular control. When the fry were stimulated with a glass rod, they seemed unable to swim away and responded only by weak contortions of the body, such as lifting the head and tail away from the bottom of the aquarium. There was wide individual variation in the time lapse before these signs of stress appeared in the fry, and there seemed to be little correlation between concentration of DDT and the time by which all the fish in a tank appeared affected. Within 7 days, all survivors exhibited the uncontrolled muscular movements. At the end of 14 days, the control fry had resorbed their yolk sacs almost entirely and had become pigmented, while the fry in DDT remained pale and still retained the large yolk sacs--an indication that little development had occurred in the 2-week exposure to DDT.

One to 14-day TL/m determinations for 10-week trout fry: -- After the fry had resorbed their yolk sacs, they became sensitive to exceedingly small dosages. The 14-day TL/m value for the 10-week fry was approximately 1/300 as great as the value for the 2-week fry. The appearance and duration of stress symptoms before death appeared later and lasted longer in the lower concentrations of DDT. A change in pigmentation was the first indication of stress. The fry became much lighter except for the tail tip, which was very dark. Disturbances of nervous and muscular control were indicated first by quick jerky movements, followed by complete loss of balance. Just before death, the fry were found lying on their backs, the mouths operated with apparent difficulty, and there was no response to probing with a glass rod.

One to 14-day TL/m determinations for number one fingerlings: -- After the trout had grown to the fingerling stage, they were more resistant to DDT than the 10-week fry which had recently lost the yolk sacs. None of the fish in .032 ppm survived more than 2 days, but of the fish in .010 ppm, 70 percent survived 14 days of exposure and 100 percent of the fish in the lower concentrations of .0032, .0010, and .00032 survived the 2-week test. On the basis of the data, the 7- and 14-day TL/m was determined to be .014 ppm DDT (table 4). The 14-day TL/m for these fish was found to be 25 times more than for the 10-week fry.

Histopathological condition of trout fry and adult guppy tissues following exposure to DDT

In general, histopathological conditions were similar in the tissues of both the trout and guppies despite the differences in age, DDT concentration, and exposure periods. Consequently, the description of tissue changes caused directly or indirectly by DDT is presented as that which is typical either of the experimentals or of the controls.

Brain: -- No histopathological condition was observed in the mid- or hindbrain of the guppy or trout. A few very small areas of vacuolation, perhaps indicative of irregularly swollen myelin sheaths or degenerating and swelling oligodendroglia, were present in the outer portion of the cerebral lobes of one trout fry.

Intestine: -- Marked abnormalities were present along the intestinal tract of both fish. In the guppy both the connective tissue and columnar epithelium were necrotic and highly disorganized (figs. 3 and 4). In most villi it was not possible to distinguish whether the cells of the epithelial layer had disappeared or dispersed. The changes in the trout intestine were even more marked. The connective tissue between the villi and the smooth muscle was either necrotic or entirely absent (figs. 5 and 6). The columnar cells were irregular as in the gut of the guppy. There was also severe vacuolation of the epithelium (fig. 7). This necrotic layer was occasionally observed sloughing off into the lumen.

Liver: -- Degeneration of varying degrees was noted in the liver tissue. In the extreme cases in the guppy liver, necrosis gave the entire organ a foamy appearance (figs. 8 and 9). Both the normal and the experimental trout livers were full of small vacuoles (figs. 10 and 11), perhaps fat vacuoles resulting from the resorption of the fatty yolk. Mount[4/] also found fatty vacuolation in the livers of normal and experimental guppies in endrin and attributed it to a high content of fat in the diet. There was some enlargement of the vacuoles in the livers of the experimental fish, and swollen nuclei were scattered in the liver of the DDT-exposed trout.

Kidney: -- There was no visible change in the guppy kidney tubules by the first or second day, but the tubules of the trout were generally occluded or congested with debris. There was some sloughing of the epithelial cells of the tubules (fig. 12).

4/ Mount, D. Personal communication of October 31, 1960, to Susan King from Taft Sanitary Engineering Center. (Thesis-1960. A study of the chronic effects of endrin, an insecticide, on the guppy /Lebistes reticulatus/, and on the bluntnose minnow /Pimephales notatus/, Ohio State University.

Adrenal tissue: -- The adrenal tissue of the bony fish is characteristically scattered as epithelial whorls in the kidney tissue Brown, 1957; Andrew, 1959). The solid, epithelial masses of cells found in the kidney of the trout and guppies and tentatively identified as adrenal tissue were markedly affected by exposure to DDT. Necrosis of this tissue (more severe in the guppies than in the trout) resulted in a foamy appearance (figs. 14 and 16) in contrast to the solid structure characteristic of the normal animals (figs. 13 and 15). In the kidney of the experimental trout fry, the only region with adrenal-like epithelial cells was in the middle portion around the central blood vessel (fig. 16). The cells, though in a disorganized state, looked healthy, and their position near the blood vessel was evidence either of recovery or of incomplete destruction originally.

Macroscopic observations of the guppy spleen: -- In dissection of adult guppies killed in DDT, it was noted that the spleens were generally much smaller than in the normals, and the bright red color of stored red blood cells was present only in spots or absent entirely. A depletion in the red blood cell content of the spleen was found by Baxter (1959) in lambs poisoned with aldrin and attributed to the withdrawal of red blood cells from the stock in the spleen to replace cells lost through congestion in the lungs and hemorrhagic conditions throughout the body.

DISCUSSION

Evaluation of bioassay data

The bioassay procedure as applied to long-term experiments of chronic exposure is not entirely reliable. Few similar tests have been conducted previously, and a standardized long range procedure has not been developed. The concentration of DDT probably does not remain constant, and it is assumed that the DDT was gradually removed from the water, by adsorption onto the glass, concentration in the fish tissues, and perhaps lost to some extent in the filtered organic wastes (Garner, 1957). As the tests were intended to simulate what might occur after one spraying of DDT in the field, it was not desirable to apply a continuous flow of DDT test solution. The data do not represent fish under constant exposure to a specific concentration, but rather fish in water treated once with a certain concentration of DDT. Doudoroff (1953) also did not renew the test solutions for 10-day bioassays. A comparison of the laboratory stock solution two months after its preparation with a freshly made solution on adult guppies showed no difference in toxicity of the two solutions.

There was close correlation of the TL/m values determined in the 14-day tolerance tests on adult guppies with those reported by Henderson et al., (1959a). The deviation from Henderson's value of .028 ppm for the 7-day TL/m for guppies in soft water was -.009 ppm or 13 percent lower (.0195 ppm). The test with a second and obviously more resistant strain yielded a value greater than .032 ppm as the 7-day TL/m. This variation in values indicates the fallacy of designating any specific concentration as the TL/m. One may specify a certain range of concentrations as having a known toxicity, however, such as .032 ppm to .018 ppm for the 7-day TL/m for guppies. Before designating a safe concentration for specific fishes in a locale to be treated with an insecticide, it would be imperative to conduct a preliminary test on a representative fish sample in the particular receiving water.

There seems to be no immediate explanation for the increased resistance to DDT once fish have been exposed to sublethal doses. As indicated by the histopathological conditions discussed later, the action of DDT seems to have only a destructive effect on the tissues and a later increase in dosage would only accelerate tissue necrosis.

The 30-day test of chronic exposure to DDT in dosages sublethal to guppies at 14 days demonstrated that the TL/m value determined for 14 days (.018 ppm) applies to even longer periods of exposure under the same conditions. The loss of the fish which occurred twice after a 30-day period in the original test water might possibly be avoided by completely replacing the test solutions with fresh DDT solutions, perhaps

as often as every two weeks. Though Mount[5/] advised a continual flow of the treated water, this procedure would not provide data representing the results of one dosage of a specific concentration on the test fish. Although the cause of the deaths might be a highly contagious disease, this seems unlikely, particularly since sterilized equipment was used in the second experiment. It seems most likely that the deaths resulted from an increasingly high concentration of dissolved nitrogenous wastes which were not removed by filtering.

Though relatively few guppies were born in the test aquaria, the survival rate was somewhat reduced for those fish born in DDT solutions. These DDT-exposed fish were generally less mature at the time of birth as indicated by the distended yolk sacs. The correlation of failure of yolk resorption and hypoactivity of the adrenal gland will be discussed later. The slight, but perhaps significant, difference in length between the fry born in DDT water and those born in untreated water is attributed to a failure of the previously DDT-exposed fry to recover completely in a 40-day period. The only literature on growth effects is that of Linduska and Surber (1948) who reported no apparent inhibition of growth of oysters in DDT treated beds.

Mount[6/] found that guppies generally failed to have more than one litter while in endrin. It was not possible in the short period of my tests to determine if DDT had a similar effect. Data on quail egg and chick survival support the assumption that insecticides have a harmful effect on fish and wildlife reproduction.

For practical application, one must compare the susceptibility of other fishes relative to that of guppies before deducing concentrations safe for other species. The 14-day bioassay with a local fish, the brown trout fry and fingerlings, revealed a marked difference in susceptibility to DDT. Once the young trout fry were feeding, they became much more sensitive to DDT than were the young guppies. While dosages above .0024 ppm were sublethal to the young guppies, any concentration above .00056 ppm proved lethal to 50 percent of the trout fry at 10 weeks of age. It seems probable that water is not brought into the intestine of the young fry until they begin to feed, which is normally at about the fourth or fifth week. Once the treated water enters the intestine, the DDT can enter the body quickly by way of the blood, thus accounting for the sudden increased sensitivity. However, after a period of growth to the first fingerling stage, the trout became much more resistant to DDT than when they first began feeding. In a short bioassay on number five fingerlings placed in .10, .0056, and .0032 ppm that 0 percent, 50 percent, and 50 percent, respectively, survived a 2-week exposure. The trout fingerlings thus appeared to be only slightly more sensitive than the adult guppies to DDT (.014 ppm for the 14-day TL/m for trout compared with .018 ppm for guppies), while the feeding trout fry were exceedingly sensitive in comparison (.00056 ppm TL/m).

While it was not possible to test the sensitivity of adult trout under exacting laboratory conditions, the trend toward decreased sensitivity displayed by the finerlings makes it seem likely that adult trout, like adult guppies, are more resistant than the fry to DDT.

It is the ultimate hope that effects of field applications of insecticides on fish can be predicted in advance, by the use of standard laboratory tests. Accumulation of TL/m values under standardized conditions, such as those values determined in the present study, is the first step in realizing this hope. Application of laboratory findings to field conditions involve major but not impossible difficulties. Close approximations of safe insecticide dosages may be obtained by short bioassays on the fish from the water area involved, using the receiving water for the test solutions to which the DDT concentrations are added in the same formulation as that to be used in the field. A comparison of the short bioassay data with results obtained after longer exposures under standarized conditions, such as the values obtained in the survey

5/ See footnote 4
6/ See footnote 4

of Henderson et al., (1959a, b) or in the present study, would provide a basis for determining a safe application level. Factors which might affect the toxicity of a field dosage, such as a possible interaction of DDT with organic and inorganic materials, any variation in volume of water per fish, stress situations, rainfall, or contaminated runoff from insecticide treated area (Young et al., 1951) should be considered before application of the insecticide.

The removal of food may actually prove to be of more imminent danger to fish than the toxicity of the insecticide, and evaluation of effects of DDT might better be based on invertebrate food organisms rather than on fish. DDT and other chlorinated hydrocarbons are known to be toxic to lower organisms in the fish food chain, though specific TL/m values have not been determined (Harrington et al., 1958). It requires only an ounce or two of DDT per acre to kill crustaceans (Leedy)[7/]. The microfauna, especially the protozoa, are relatively resistant to the chlorinated hydrocarbons (DeWitt et al., 1960). Even if over 50 percent of the lower food-chain organisms survived, the dead ones might be eaten by fish. Though DeWitt and George (1960) report no harmful effects on fish from eating insects killed by DDT sprayings, Hoffman (1959) and Janzen (1960) report fish affected by insects sprayed with DDT. The amount of poisoned insects required to kill fish varied considerably, however.

It is obvious that use of standardized procedures in the laboratory results in comparable, reliable, and reproducible data, but cannot reproduce conditions of a particular natural situation, which are never constant or identical with other natural situations.

Histopathological conditions

Although bioassays with DDT are necessary for estimation of fish survival after field application, it seems of at least equal importance to determine the physiological cause of death. Some research concerning effects of the DDT on the tissues of exposed animals is now in progress in several laboratories. Janzen (1960) has reported that pesticides are concentrated in fish, especially in the fatty tissues. Damage of liver and kidney tissues, reduction of red blood cell production, depressed growth rates, and reduced efficiency of reproduction may result. These conditions would suggest increased sensitivity to diseases. Janzen pointed out that under stress, such as lack of food, the DDT stored in the fat is likely to be released into the system. The effects of such a release are not yet known. Cope (1959) reported storage of chlorinated hydrocarbons in the kidney, pyloric caecum, and brain, but none in the liver. In other chemical bioassays conducted at his laboratory, DDT has been found in tissues two years after exposure of trout (up to .94 ppm DDT in the tissues) and whitefish (0.7 ppm DDT and 1.2 ppm DDE).

The determination of DDT concentrations in tissues or even test solutions is difficult, and no method has yet been established that is both rapid and accurate. An elaborate paper chromatographic analysis for DDT was proposed by Mitchell (1954). The analysis is based on subjective color comparisons with a standard and is, therefore, subject to some error. Cope's results mentioned above stem from a complex biochemical analysis of tissues requiring special equipment and skilled technique. This procedure is still being perfected. Amounts of stored insecticide in fish tissue depend on the compound and species. Small amounts seem to be of no harm to the fish. This is indeed fortunate as every assayed fish, even from supposedly uncontaminated waters, at the Denver Laboratory has had some DDT in the tissues. Mount[8/] found high concentrations of endrin in the liver, intestine, spleen, and kidney of carp after 2 - 7 days of exposure. Similar and more extensive assays have been performed on quail and pheasant (Anon., 1951; DeWitt, 1955; DeWitt et al., 1960) and the chlorinated hydrocarbons have been found to accumulate in the tissues of birds also, especially in the fat and muscle.

7/ Leedy, D. L. November 15, 1960 Draft report on (1) wildlife values and (2) pesticide usage in conservation programs. U. S. Fish and Wildlife Service, U. S. Department of the Interior

8/ See footnote 4.

Techniques to determine interaction of chlorinated hydrocarbons with a specific enzyme or metabolic pathway in organisms exposed to toxicants are being developed by Weiss (1960). This investigator cites similar work of Hosein who has found that a shift in a metabolic pathway was affected, leading to an increased production of carnitine, which accumulates in the brain and interferes with nerve function, resulting in convulsive activity.

The histopathological findings for the fish exposed to DDT show close agreement with the results of other workers using different test animals and chlorinated hydrocarbons. Though brain lesions have been seen in the cerebral lobes in the dog (Kitselman, 1953) after aldrin and dieldrin poisoning and sometimes in lamb and poultry after aldrin poisoning (Baxter, 1959), there are other cases cited by Baxter, in which insecticide poisoning has not produced brain lesions. This is true for sheep exposed to dieldrin and occasionally for the lamb. The absence of lesions also in the trout and guppy forebrains indicates that brain lesions may not be a significant cause of death in insecticide poisoning.

No histological studies of the intestine of animals exposed to insecticides were found in the literature, though Gowdey and Stravraky (1955) mentioned that dieldrin and aldrin had inhibitory effects on intestinal motility. The severity of the intestinal lesions found in the trout and guppies may have been a direct cause of death in the fish by preventing the normal digestion and assimilation of food. If the disturbances in the intestine are an indirect cause of death, through starvation, the insensitivity of the trout to DDT before the yolk sacs are resorbed could be explained. However, further study is needed to determine whether the fish are resistant because the mouth does not operate to bring water into the intestine at this stage in development, thus preventing absorption of DDT into the blood stream (as suggested by Mount[9/], or because a normally functioning intestine is not needed while the yolk sac is still present.

Nelson et al., (1949) using DDD, Kitselman (1953) using dieldrin and aldrin on dogs, and Baxter (1959) studying effects of aldrin on lambs all found moderate to severe fatty degeneration in the liver after exposure to the insecticide. The indications of degeneration in the lamb liver were necrosis of the hepatic cells, accumulation of refractile round bodies which looked similar to vacuoles in hematoxylin-eosin, bleeding, and general congestion. The liver in the dogs was found in the severest cases to be quite foamy in appearance. A more complete histological study series of the test fish might reveal recovery potential of necrotic livers and at what stage in the necrotic process the swollen cells which were seen in the trout liver appear. Waud (1952, as reported by Gowdey et al., 1955) found that the blood sugar of cats fed aldrin doubled and that this level was even higher when convulsions set in before death. The lethal dosage of DDT given the guppies (.032 ppm) may have had a similar effect, causing a complete conversion of the stored glycogen in the liver to glucose.

Bell (1961), Kitselman (1953), and Baxter (1959), using different animals and insecticides reported that chlorinated hydrocarbons caused degeneration of the renal tubules. The severity of degeneration varied with the animal and concentration and ranged from an increase in tubular fat and slight hemorrhaging in the surrounding tissue to eventual occlusion of the tubules with debris and sloughing of necrotic epithelium into the lumen. Although there was no noticeable fatty degeneration in the tubules of the trout or guppies, marked necrosis of the epithelium had occurred in many of the trout fry tubules after two weeks.

Though both interrenal and chromatin cells of the adrenal gland are scattered in the bony fish, both were seen best in the whorls of epithelial cells in the kidney of both the trout and guppy. Degeneration of this tissue in the fish was indicative that the adrenal tissue was directly affected by the DDT.

The role of the adrenal gland in the effects of DDT on an organism is implicated in

9/ See footnote 4.

connection with yolk sac retraction and differentiation of the intestine. By various experiments on the chick embryo, Mogg (1953; Moog et al., 1955) has demonstrated that glucocorticoids, hormones secreted by the zona fasciculata of the adrenal gland, accelerate the retraction of the yolk sac in chicks and the later stages of differentiation of the duodenal mucosa. She did not propose a definite mode of action of the adrenal cortical hormone, though she suggested an indirect effect on phosphatase activity and possibly other enzymes.

The various kinds of evidence seem to indicate that DDT has a direct inhibitory and destructive effect on the adrenal cortical tissue and that other effects are secondary. The destruction of adrenal tissue of the fish exposed to DDT and the atrophy of the zona fasciculata found by others in animals exposed to various chlorinated hydrocarbons might be considered primary effects leading to the secondary effects such as those found in the kidney, liver, and intestine. The hyperexcitability and loss of muscular control, the failure of trout yolk sac resorption, the vacuolation in the liver, and the deterioration of the intestine could be immediate causes of death under extreme conditions. The major characteristics for Addison's disease, a hypoactive condition of the adrenal gland in man, are similar for animals exposed to the chlorinated hydrocarbons. Pigmentation is affected, and there are gastro-intestinal disturbances, weaker muscles, hypoglycemia, and some effects on reproduction.

SUMMARY AND CONCLUSIONS

Fourteen day bioassays were conducted on young and adult guppies by exposing them to serial logarithmic dilutions of DDT under controlled conditions. The values at which 50 percent of the fish survived (TL/m) in 1, 4, 7 and 14 days were determined. On the basis of these assays, it was determined that young guppies two to three weeks of age are approximately 10 times more sensitive to DDT than the adults. The 14-day TL/m for the young was .0024 ppm and, though the TL/m varied slightly with different strains of fish, .018 ppm was established as the sublethal dosage for the adult fish.

Similar 14-day bioassays were conducted with young brown trout. The fry, while still depending on the yolk sac for food (two weeks old) were found to be exceedingly resistant to DDT. Over 50 percent were able to withstand .18 ppm DDT in a 14-day period. Once the yolk sac was resorbed, the fish became very sensitive to the insecticide. The 14-day TL/m for 10-week-old fry was .00056 ppm and .014 ppm for the number one fingerlings.

The 14-day TL/m for adult guppies (.018 ppm DDT) was sublethal to adult guppies over an extended exposure of 30 days. It was postulated that exposed fish would not be harmed seriously in DDT solutions of a concentration below the 14-day TL/m.

Limited observations on growth and reproduction indicated that exposure to DDT did not prevent reproduction in guppies in the first 30 days of chronic exposure, but many of the guppies born in DDT were dead or died within several hours. The survival rate was lower for those born in DDT than for those born in untreated water. The data, however, were not sufficient to make definite conclusions. Observations in the literature indicate that after the first litter, insecticides will inhibit reproduction. The cause of the death of guppies in aquaria kept under controlled conditions needs to be ascertained before the effects of one dosage of DDT on reproduction can be determined.

Guppies born in DDT, especially in concentrations of .010 and .0185, had abnormally large yolk sacs. The sacs were resorbed in several days if the fry survived. Guppies born in DDT and removed to untreated water were slightly shorter after 40 days than guppies born in the control and treated similarly (averages of 10 mm and 11 mm respectively), though the data are inconclusive.

Exposure of the guppies to sublethal dosages of DDT for 14 days and then removal to a concentration toxic to normal fish within three days -- .032 ppm, demonstrated that the fish had increased resistance to the toxicant and the dosage in most cases was no longer lethal. It was found that the greatest resistance

developed in the fish first exposed to .010 and .0056 ppm DDT.

The physical signs of stress exhibited by fish in DDT, include hyperexcitability and loss of muscular control, the presence of yolk sacs in the gupples born in DDT, the failure of the sac resorption in trout fry exposed to DDT, and various histopathological conditions found in the tissues. This syndrome suggests that DDT has a direct effect on adrenal tissue, especially that portion secreting the glucocorticoids. The most marked histopathological conditions in the fish attributed to the DDT were found in the liver, the intestine, and the adrenal-like tissue in the kidney. Modification of the kidney tubules and the gross appearance of the spleen were occasionally observed.

There is a very serious need for extensive study of the chlorinated hydrocarbons and their effects on fish survival, reproductive potential, and physiological condition as exhibited by the tissues. There are many factors in the field which modify bioassay determinations. It is likely that the safe dosages of insecticides for fish will be less than that necessary for insect control. If such is found to be the case, recourse to other means of control of the insects will be necessary to prevent extensive loss of fish.

LITERATURE CITED

Adams, L., M. G. Haravan, N. W. Hosley, and D. W. Johnston.
1949. The effects on fish, birds, and man of DDT used in control of forest insects in Idaho and Wyoming. Journal of Wildlife Management, vol. 13, p. 245-254.

Andrew, W.
1959. Textbook of Comparative Histology. Oxford University Press, New York, chap. 13, p. 527 - 530.

Anonymous.
1951. Insecticide storage in adipose tissue. Journal of the American Medical Association, vol. 145, p. 735 - 736.

Anonymous.
1960. A. I. E. C. holds pesticide symposium. Agricultural Chemicals, vol. 15, p. 8.

Baxter, J. T.
1959. Some observations on the histopathology of aldrin poisoning in lambs. Journal of Comparative Pathology and Therapeutics, vol. 69, p. 185 - 191.

Bell, J. T., Jr.
1961. Histological and histochemical effects of DDD on goat adrenals, pituitaries, livers, and kidney. American Association of Anatomists, Anatomical Record, vol. 139, p. 207.

Brown, M. E.
1957. The Physiology of Fishes. Academy Press, Inc., New York, vol. 1, chap. 6, p. 252 - 254.

Breder, C. M., Jr., and C. W. Coates.
1932. A preliminary study of population stability and sex ratio of Lebistes. Copeia, p. 147 - 155.

Clawson, S. G.
1958. Wild turkey populations on an area treated with heptachlor and dieldrin. Alabama Birdlife, vol. 6, p. 4 - 8. (Cited by DeWitt et al., 1960.)

Cope, O. B.
1959. The retention of DDT by trout and whitefish. Biological Problems in Water Pollution, U. S. Department of Health, Education and Welfare.

Cope, O. B., C. M. Gjullin, and A. Storm.
1947. Effects of some insecticides on trout and salmon in Alaska with reference to blackfly control. Transactions American Fisheries Society, vol. 77, p. 160 - 177.

Cottam, C. and E. Higgins.
1946. DDT: Its effects on fish and wildlife. U. S. Department of the Interior, Fish and Wildlife Service, Circular 11, 14 p.

Davidow, B. and F. J. Sabatino.
1954. Biological survey test for chlorinated insecticides. Journal of Association of Official Agricultural Chemists, vol. 37, p. 902 - 905.

DeWitt, J. B.
1955. Effects of chlorinated hydrocarbon insecticides upon quail and pheasants. Journal of Agricultural and Food Chemistry, vol. 3, p. 672 - 676.

1956. Chronic toxicity to quail and pheasants of some chlorinated insecticides. Journal of Agricultural and Food Chemistry, vol. 4, p. 863 - 866.

1957. H-bomb in the pea patch. Wildlife in North Carolina, vol. 21, p. 1-4.

DeWitt, J. B. and J. L. George
1960. Bureau of Sport Fisheries and Wildlife--Pesticide Wildlife Review: 1959. U. S. Department of the Interior, Fish and Wildlife Service, Circular 84, 36 p.

Doudoroff, P., B. G. Anderson, G. E. Burdick, P. S. Galtsoff, W. G. Hart, R. Patrick, E. K. Strong, E. W. Surber, and W. M. Van Horn.
1951. Bioassay methods for the evaluation of acute toxicity of industrial wastes to fish. Sewage and Industrial Wastes, vol. 23, p. 1380 - 1397.

Doudoroff, P., M. Katz, and C. M. Tarzwell.
1953. Toxicity of some organic insecticides to fish. Sewage and Industrial Wastes, vol. 25, p. 840 - 844.

Ellis, M. M.
1937. Detection and measurement of stream pollution. U. S. Bureau of Fisheries Bulletin 48, p. 365 - 437.

Farris, E. J.
1950. The Care and Breeding of Laboratory Animals. John Wiley and Sons, Inc., New York, chap. 4, p. 345-427.

Fischer, C. D.
1956. Pesticides: past, present, and prospect. Chemical Week, October 27, p. 59 - 90; November 3, p. 52 - 88.

Foster, A. C.
1951. Some plant responses to certain insecticides in the soil. U. S. Department of Agriculture, Circular 862, p. 1 - 41. (Cited by Surber, 1951).

Garner, R. J.
1957. Veterinary Toxicology. Bailliere, Tindall and Cox, London, chap. 4, p. 205 - 231.

George, J. L.
1959. Effects on fish and wildlife of chemical treatments of large areas. Journal of Forestry, vol. 57, p. 250 - 254.

George, J. L., R. F. Darsie, Jr., and P. F. Springer.
1957. Effects on wildlife of aerial applications of strobane, DDT, and BHC to tidal marshes in Delaware. Journal of Wildlife Management, vol. 21, p. 42 - 53.

Gibson, M. B. and B. Hurst.
1955. The effect of salinity and temperature on the preadult growth of guppies. Copeia, p. 241 - 243.

Ginsberg, J. M. and D. M. Jobbins.
1954. Research activities on chemical control of mosquitos in New Jersey in 1953. Proceedings of the New Jersey Mosquito Extermination Association, vol. 42, p. 270 - 291.

Gordon, M.
1955. Guppies as pets. Aquarium, New York Zoology Society, 32 p.

Gowdey, D. W. and G. W. Stravraky.
1955. A study of the autonomic manifestations seen in acute aldrin and dieldrin poisoning. Canadian Journal of Biochemistry and Physiology, vol. 33, p. 272 - 282.

Harrington, R. W., Jr. and W. L. Eidlingmayer.
1958. Effects of dieldrin on fishes and invertebrates of a salt marsh. Journal of Wildlife Management, vol. 22, p. 76 - 82.

Hart, W. B., P. Doudoroff, and J. Greenbank.
1945. The Evaluation of the Toxicity of Industrial Wastes, Chemicals, and other Substances to Fresh-water Fishes. The Atlantic Refining Company, Philadelphia.

Henderson, C., Q. H. Pickering, and C. M. Tarzwell.
1959a. Relative toxicity of ten chlorinated hydrocarbon insecticides to four species of fish. Transactions American Fisheries Society, vol. 88, p. 23 - 32.

1959b. The toxicity of organic phosphorus and chlorinated hydrocarbon insecticides to fish. Transactions 2nd Seminar on Biological Problems in Water Pollution, U. S. Department of Health, Education, and Welfare, April, 13 p.

Henderson, C. and C. M. Tarzwell.
1957. Bioassays for control of industrial effluents. Sewage and Industrial Wastes, vol. 29, p. 1002 - 1017.

Hoffman, C. H.
1959. Are the insecticides required for insect control hazardous to aquatic life? Biological Problems in Water Pollution, U. S. Department of Health, Education, and Welfare.

Ingram, W. M. and C. M. Tarzwell.
1954. Selected bibliography of publications relating to undesirable effects upon aquatic life by algicides, insecticides, and weekicides. U. S. Department of Health, Education, and Welfare, Public Health Service, Publication No. 400, 28 p.

Janzen, D. H.
1960. Problems with fish and pesticides in the United States. U. S. Department of the Interior, Fish and Wildlife Service. (International Union for the Protection of Nature, Warsaw, Poland. June.)

Keenleyside, M. H. A.
1959. Insecticides and wildlife. Canadian Audubon, vol. 21, p. 1 - 7.

Kerswill, C. J. and P. F. Elson.
1955. Prelminary observations on effects of 1954 DDT spraying on Miramichi salmon stocks. Progress Report of the Atlantic Coast Station of the Fishery Reserve Board of Canada, vol. 62, p. 17 - 23.

Kitselman, C. H.
1953. Long term studies on dogs fed aldrin and dieldrin in sublethal dosages, with reference to the histopathological findings and reproduction. Journal of American Veterinary Medical Association, vol. 123, p. 28 - 30.

Lawrence, J. M.
1950. Toxicity of some new insecticides to several species of pondfish. The Progressive Fish-Culturist, vol. 13, p. 141 - 146.

Leffler, R. L.
1958. Letter of May 1, 1957, to the Honorable H. C. Bonner, Chairman on Merchant Marine and Fisheries. House of Representatives, 85th Congress, 2nd Session, Report No. 2181.

Linduska, J. P. and E. W. Surber.
1948. Effects of DDT and other insecticides on fish and wildlife--Summary of investigations during 1947. U. S. Department of the Interior, Fish and Wildlife Service, Circular 15, 19 p.

Long, C. N. H., B. Katzin, and E. Fry.
1940. The adrenal cortex and carbohydrate metabolism. Endocrinology, vol. 26, p. 309 - 344.

Mitchell, C.
1954. Separation and identification of chlorinated organic pesticides by paper chromatography VI. Tech. BHC, lindane, DDT, rhothane. Journal of Association of Official Agricultural Chemists, vol. 37, p. 996 - 1001.

Moog, F.
1953. The functional differentiation of the small intestine. III. The influence of the pituitary adrenal system on the differentiation of phosphatase in the duodenum of the suckling mouse. Journal of Experimental Zoology, vol. 124, p. 329 - 346.

Moog, F. and D. Richardson.
1955. The functional differentiation of the small intestine. IV. The influence of adrenocortical hormones on differentiation and phosphatase synthesis in the duodenum of the chick embryo. Journal of Experimental Zoology, vol. 130, p. 29 - 56.

Nelson, A. L., and E. W. Surber.
1947. DDT investigations by the Fish and Wildlife Service in 1946. U. S. Department of the Interior, Fish and Wildlife Service, Special Scientific Report--Fisheries No. 41, 8 p.

Nelson, A. A. and G. Woodard.
1949. A. M. A. Archives of Pathology, vol. 48, p. 387 - 294.

Rudd, R. L. and R. E. Genelly.
1956a. Chronic toxicity of DDT, toxaphene, and dieldrin to ring-necked pheasants. California Department of Fish and Game, Game Bulletin, vol. 42, p. 5 - 14.

1956b. Effects of DDT, toxaphene and dieldrin on pheasant reproduction. Auk, vol. 73, p. 529 - 539.

Shepard, H. H.
1956. The pesticide situation for 1955 - 56. Commodity Stabilization Service, U. S. Department of Agriculture, 17 p.

Shepard, H. H., J. N. Mahan, and C. A. Graham.
1959. The pesticide situation for 1958-59. Commodity Stabilization Service, U. S. Department of Agriculture, 24 p.

Silliman, R. P.
1948. Factors affecting population levels in Lebistes reticulatus. Copeia, p. 40 - 47.

Silliman, R. P., J. S. Gutsell, C. E. Dunbar, and S. B. Friddle.
1958. Experimental exploitations of fish populations. U. S. Department of the Interior, Fish and Wildlife Service Fishery Bulletin 133, vol. 58, p. 215-252.

State Water Pollution Control Board.
1952. Propagation of fish and aquatic life. Water Quality Criteria, p. 156-158.

Surber, E. W.
1947. Annual report for the fiscal year 1947. U. S. Fish and Wildlife Service, Branch of Fishery Biology, 114 p. (mimeo.)

1951. Toxicities of some chemical substances to fish. Pollution Abatement Conference, Manufacturing Chem. Association, Inc., vol 0, p. 35 - 45.

Tarzwell, C. M.
1950. Effects of DDT mosquito larviciding on wildlife. V. Effects on fishes of the routine manual and airplane application of DDT and other mosquito larvicides. U. S. Department of Health, Education, and Welfare, Public Health Service, vol. 65, p. 231 - 255.

1957a. The use of bioassays in relation to the disposal of toxic wastes. Proceedings 3rd Ontario Industrial Waste Conference for 1956 - 1957.

Tarzwell, C. M.
1957b. Water quality criteria for aquatic life. Transactions Seminar on Biological Problems in Water Pollution, U. S. Department of Health, Education, and Welfare, April, 1956, 1957, p. 246 - 272.

1958. The toxicity of some organic insecticides to fishes. Proceedings of the 12th Annual Conference of the Southeastern Association of Game and Fish Commissioners, p. 233 - 239.

Tarzwell, C. M. and C. Henderson.
1957. Toxicity of dieldrin to fish. Transactions American Fisheries Society, vol. 86, p. 245 - 257.

Turner, C. L.
1937. Reproductive cycles and superfetation in poeciliid fishes. Biological Bulletin, vol. 72, p. 145 - 164.

U. S. Fish and Wildlife Service.
1960. Care of tropical aquarium fishes. U. S. Department of the Interior, Fish and Wildlife Service, Fishery Leaflet 411.

U. S. Public Health Service.
1956, 1957. Bioassay Investigations for International Joint Commission. U. S. Department of Health, Education, and Welfare.

Warren, C. E. and P. Doudoroff.
1958. The development of methods for using bioassays in the control of pulp mill waste disposal. Technical Association of the Pulp and Paper Industry, vol. 41, no. 8, p. 211A - 216A.

Waud, R. A.
1952. Journal of Pharmacology and Experimental Therapeutics, vol. 106, p. 423. (Cited by Gowdey et al., 1955).

Weiss, C. M.
1960. Personal communication from Acting Head of Department of Sanitary Engineering, University of North Carolina, October 6.

Young, L. A. and H. P. Nicholson.
1951. Stream pollution resulting from the use of organic insecticides. The Progressive Fish-Culturist, vol. 13, p. 193 - 198.

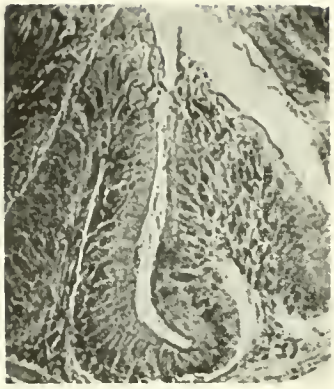

Figure 3

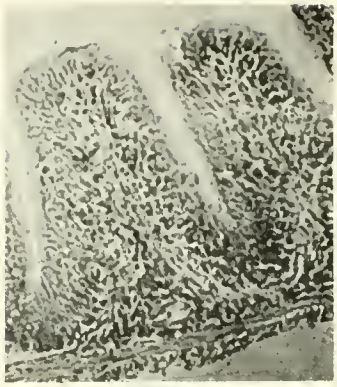

Figure 4

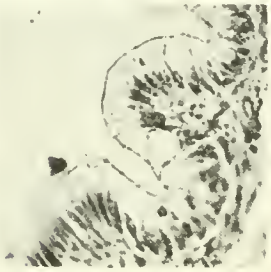

Figure 5

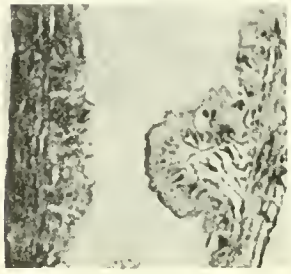

Figure 6

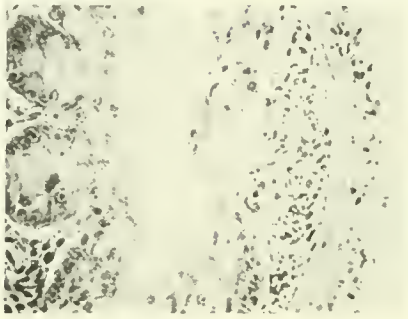

Figure 7

Explanation of Figures

Photomicrographs of the intestine of 4-week trout fry (yolk sac still present) and adult guppies; fixed in Bouin's fluid, embedded in paraffin, sectioned at 8 μ and stained in Hematoxylin-Eosin. X 435

Figure 3--Intestine of normal guppy showing well-defined columnar epithelium and connective tissue.

Figure 4--Intestine of guppy in .032 ppm DDT for two days showing disorganization and necrosis of columnar epithelium and connective tissue. Nuclei of the epithelial layer appear indistinguishable from nuclei of the connective tissue.

Figure 5--Intestine of normal trout fry showing organized columnar epithelium, a few goblet cells, and normal connective tissue.

Figure 6--Intestine of trout fry in 10 ppm DDT for 14 days showing absence of connective tissue below columnar epithelium in parts. Necrosis of remaining connective tissue in villus is marked.

Figure 7--Intestine of trout fry in 3.2 ppm DDT for 14 days showing severe vacuolation and absence of organization of columnar epithelium. Connective tissue did not appear to be affected in this trout.

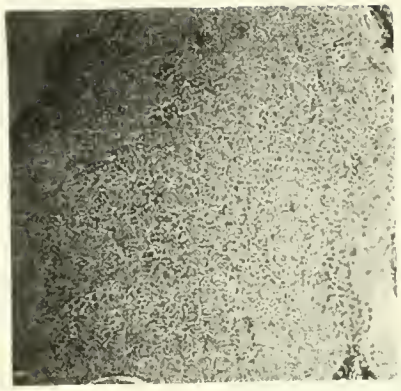

Figure 8

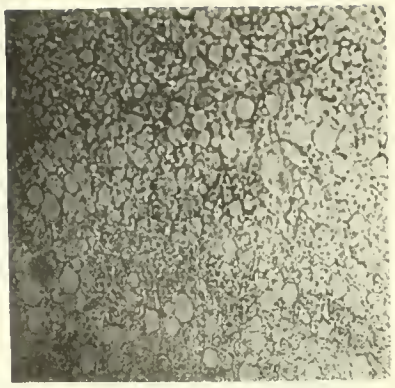

Figure 9

Figure 10

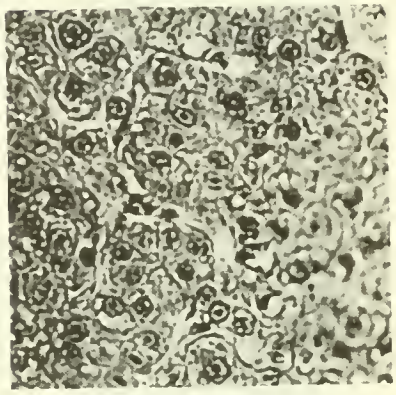

Figure 11

Explanation of Figures

Photomicrographs of the liver of 4-week trout fry (yolk sac still present) and adult guppies; fixed in Bouin's fluid, embedded in paraffin, sectioned at 8 μ and stained in Hematoxylin-Eosin.

Figure 8--Liver of normal guppy in which liver cells are solidly packed. X 147

Figure 9--Liver of guppy in .032 ppm DDT for one day showing foamy appearance due to severe vacuolation and necrosis of entire liver. X 147

Figure 10--Liver of normal trout fry showing even distribution of small vacuoles, perhaps fat vacuoles resulting from resorption of the fatty yolk. Nuclei all appear to be about the same size. X 435

Figure 11--Liver of trout fry in 3.2 ppm DDT for one day showing vacuoles as in the normal though some are possibly larger. Large irregularly swollen nuclei are scattered throughout the liver. X 435

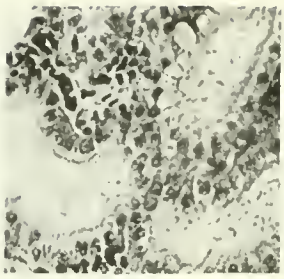

Figure 12

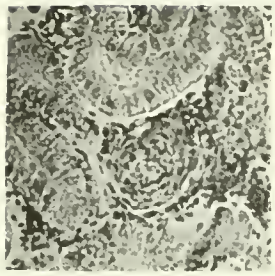

Figure 13

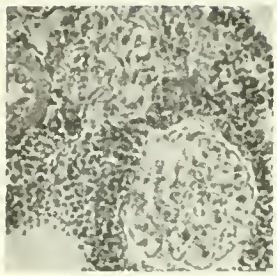

Figure 14

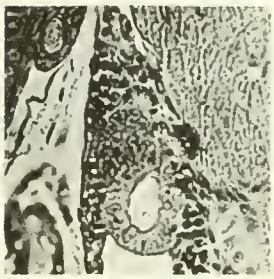

Figure 15

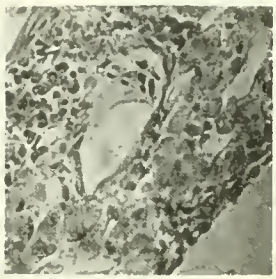

Figure 16

Explanation of Figures

Photomicrographs of the kidney tissue including tubules and adrenal tissue of 4-week trout fry (yolk sac still present) and adult guppies; fixed in Bouin's fluid, embedded in paraffin, sectioned at 8 μ, and stained in Hematoxylin-Eosin. X 425

Figure 12--Kidney of trout fry in 3.2 ppm DDT for 14 days showing congestion of tubules and necrosis of tubular epithelium which is sloughing off into the lumen.

Figure 13--Kidney of normal guppy showing solid whorl of epithelial cells. This adrenal tissue was found scattered throughout the kidney.

Figure 14--Kidney tissue of guppy in .032 ppm DDT for two days showing foamy appearance of whorls of epithelial cells which are in a state of necrosis and vacuolation.

Figure 15--Kidney tissue of normal trout fry showing solidly packed whorl of epithelial cells of adrenal tissue adjacent to a kidney tubule.

Figure 16--Kidney tissue of trout fry in 10 ppm DDT for 14 days showing apparently healthy or normal epithelial cells, identified as adrenal tissue, around the central blood vessel. The disorganized appearance of these cells and their position near the blood vessel indicate either recovery of adrenal tissue or incomplete destruction originally.